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Chapter 18

c0018 Evolution and Immune Function of Fish Lectins

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s0010 INTRODUCTION

p0015 The term "lectin" is commonly used to encompass a wide variety of carbohydrate-binding proteins, widely distributed in viruses, prokaryotes, and eukaryotes.[1] The first invertebrate lectins were described in the early 1900s in the snail *Helix pomatia*,[2] the horseshoe crab *Limulus polyphemus*,[3] and the lobster *Homarus americanus*.[3] Among the vertebrates, Watkins and Morgan[4] first described an L-fucose-specific lectin in the European eel *Anguilla anguilla*, which led to the discovery of the carbohydrate nature of the H blood substance.

p0020 Animal lectins are grouped in various molecular families, differing in carbohydrate recognition domain (CRD) structure and organization.[1,5–7] Based on their CRD sequence motifs and cation requirements, animal lectins can be categorized in several families, such as C-type lectins (CTLs), galectins (formerly S-type lectins), rhamnose-binding lectins (RBLs), F-type lectins (FTLs), X-type lectins (XTLs), I-type lectins, P-type lectins, and pentraxins.[1,5–7]

p0025 Lectins are involved in a variety of key biological processes ranging from development to immune responses.[1,7–11] The roles of lectin-carbohydrate interactions in self/non-self recognition in early development and innate immunity of vertebrates has been well documented.[1,7–11] In some ("chimeric" or "mosaic") lectins, the combination of one or multiple CRDs with distinct functional domains enable additional effector functions including opsonization and phagocytosis, and activation of the complement pathway.[12]

p0030 It is now well established that protein–carbohydrate interactions constitute the basis of mechanisms mediating signaling functions, cell communication, and self/non-self recognition that are critical in the establishment and maintenance of highly specific mutualistic associations in organism–microbe complexes.[1,13,14] In this respect, mutual benefit (symbiosis or commensalism)

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depends on the maintenance of a tightly regulated balance, whereas colonization of tissues beneficial to the microbe could lead to loss of host fitness (pathogenesis), unless host defense responses are able to eliminate the foreignness pentraxins.[1,13,14] Microheterogeneity, originating from multiple lectin gene copies, allelic variation, or posttranslational modifications of the gene products, expands the molecular diversity and recognition capabilities.[7,15,16]

FISH LECTINS

In fish, CTLs, FTLs, galectins, and pentraxins have been identified in both cartilaginous and bony fish.[17] In addition, selectins and other lectin genes have been found in the currently available fish genomes. Members of most lectin families described in mammals, including CTLs, XTLs, and galectins have been isolated from fish serum, skin mucus, and other tissues.[17–20] Furthermore, some lectin families unique to fish, such as the RBLs have also been identified in eggs and embryos[21] but are also present in the serum.[22] Lectins can exert opsonic activity[7,9,23] or enhance respiratory burst and bactericidal activity of phagocytic cells.[19,24,25]

Considerable heterogeneity has been identified in the FTL of the Japanese eel[15] that exists in various isoforms. The presence of isoforms has also been shown in C-reactive proteins from the serum of the Indian major carp, *Labeo rohita* in which a shift in expression from normal to diverse structural isoforms have been demonstrated.[26]

Several fish species have proved to be useful model organisms for gaining insight into structural, functional, and evolutionary aspects of lectin immunobiology.[17,27] For example, based on the identification of a novel CRD sequence motif[20] and a unique structural fold,[28] the FTL family was identified both in prokaryotes and in fluids and tissues of invertebrates and vertebrates.[29–34]

THE LECTIN REPERTOIRES IN FISH: GENOMIC, STRUCTURAL, AND FUNCTIONAL DIVERSITY

Most components of the mammalian innate and adaptive immune response are present in elasmobranchs and teleost fish. However, it is currently accepted that their innate immune responses carry a substantial burden of the defense functions against infectious diseases.[17] Evidence of the presence of lectins in teleost fish, particularly in plasma and eggs, was obtained by serological approaches. More recently, however, the implementation of biochemical, molecular, genomic, and structural approaches has contributed to the comprehensive genomic, structural, and functional characterization of the fish lectin repertoires.[17]

The determination of the lectin structures by crystallization or homology modeling using as templates lectin structures from other species have provided detailed information on the amino acid residues that interact with the ligands.[28,29] Like mammals, recent information suggests that the lectin

repertoires are diversified among teleost fish, including representatives from most of the known lectin families.[17] In addition, recent studies on teleost fish have identified novel families of lectins, some of them with members present in other vertebrate and invertebrate taxa.[1,17] This lectin diversity is greatly amplified by the presence of isoforms with differences in sugar specificity and recognition ability.[15–17] The available genomes of tetraodontid pufferfish (*Takifugu rubripes* and *T. nigroviridis*) (www.genoscope.com), zebrafish (*Danio rerio*) (www.sanger.ac.uk), and medaka (*Oryzias latipes*) (www.ensamble.org) are expanding this view even further.[17,20]

s0025 RHAMNOSE-BINDING LECTINS

p0060 More than 20 years ago,[35] a lectin with specificity for D-galactosides was identified in eggs of the sea urchin *Anthocidaris crassispina*. It was designated SUEL (sea urchin egg lectin), and it is now recognized as the first described member of a new family of animal lectins, the RBL family.[1] RBLs are Ca^{2+}-independent lectins with specificity for rhamnose and galactosides, particularly abundant in teleosts and aquatic invertebrate species, such as annelids, bivalves, and ascidians.[36,37] RBLs share the presence of one or multiple CRDs with a unique β/β fold, about 100 amino acid in length, with 8 highly conserved cysteine residues engaged in 4 disulfide bridges with characteristic topology.[22,38,39] In addition, conserved motifs (YGR, DPC, and KYL) are also found.[38]

s0030 RBLs IN FISH: BIOCHEMICAL AND MOLECULAR FEATURES

p0065 RBLs purified from fish eggs exhibited two or three tandemly repeated structures of CRD.[35,38,40–42] Three RBLs, named STL1, STL2, and STL3, were isolated from eggs of steelhead trout (*Oncorhynchus mykiss*)[41,42] and three RBLs, CSL1, CSL2, and CSL3, with a high degree of sequence identity with *O. mykiss* were isolated from chum salmon (*Oncorhynchus keta*)[43] (Table 18.1). Only one RBL (SAL) and two RBLs (WCL1 and WCL3) were isolated from catfish (*Silurus asotus*) and whitespotted charr (*Salvelinus leucomaenis*) eggs, respectively[44,45] (Table 18.1). Fish RBLs increase their expression in response to inflammatory stimuli, enhance phagocytosis acting as opsonins, and induce the synthesis and release of proinflammatory cytokines.[21,36,40,46]

p0070 The ability to recognize and bind lipopolysaccharides and lipoteichoic acid and agglutinate both gram-positive and gram-negative bacteria has been described in trout RBLs, suggesting an antibacterial activity.[22,35,44,47] In addition, RBLs have also been found in the cortex of teleost eggs as well as in the skin mucus and serum, further confirming their protective role. The ligand of fish RBL is the glycosphingolipid globotriaosylceramide (Gb3), which is abundant in membrane lipid rafts.[17,21,36] The RBL CRD appeared early in metazoan evolution and it is found in a variety of proteins, with different domain architecture,

t0010 **TABLE 18.1** Classification of RBL Family Lectins Isolated From Teleostean Fish Species

Species	Order	RBL	Type—CRD Composition (Ogawa et al.[36]; Thongda et al.[93])	Type—Sugar Specificity (Nitta et al.[96])	CRD	No. of CRD	References
Ictalurus punctatus	**Siluriformes**	**IpRBL1a**	Ia	I	▬▭▭	3	90
		IpRBL1b	Ia	I			
		IpRBL1c	Ia	I			
		IpRBL3a	IIIg	III	▬▭	2	
		IpRBL3b	IIIg	III			
		IpRBL1c	Va	–	▭	1	
Silurus asotus		**SAL**	Ia	I	▬▭▭	3	39
I. punctatus		**IfRBL**	Ia	I			91
Oncorhyncus keta	**Salmoniformes**	**CSL1**	II	II	▬▬▭	3	38
Oncorhyncus mykiss		**STL1**	II	II			37
Savelinus leucomaenis		**WCL1**	II	II			32

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O. keta		**CLS3**	IIIa	III	▬▬	2	38
O. mykiss		**STL3**	IIIa				37
S. leucomaenis		**WCL3**	IIIa				32
Dicentrarchus labrax		**DlRBL**	IIIa				2
O. keta		**CSL2**	IIIb	III	▬▬	2	38
O. mykiss		**STL2**	IIIb				37

RBLs have been classified into five groups (types I to V) based on their domain structures and the hemagglutination activity against human erythrocytes and sugar specificity against lactose.
Type I is composed of three tandemly repeated domains, while type II has two tandem-repeated domains with an extra domain. Types III and IV have two tandem-repeated domains, but they have different hemagglutination activity and sugar specificity. Type V has only one RBL domain and exists in a homodimer with a disulfide linkage between subunits.
As proposed by Thongda et al.[90] and Ogawa et al.,[25] RBLs are classified into subgroups based on their structural features of RBL-CRD compositions.
Thus RBL genes containing three domains (in an N–C orientation) were classified as type Ia and type II; genes composed of two domains were classified as a different type named IIIa, IIIb. The RBL containing only one domain was termed type Va.
CRD, carbohydrate recognition domain; *RBL*, rhamnose-binding lectin.

[AU5]

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from mammals (eg, polycystic kidney disease 1-like, axon guidance receptor EVA-1 and latrophilin) to cnidarian (rhamnospondins). RBLs together with these proteins constitute the RBL superfamily containing RBL CRDs.[48–50]

p0075 Recently, we have purified, and characterized, both biochemically and functionally, a novel RBL from sea bass, *Dicentrarchus labrax*, serum (DlRBL).[22] The purified DlRBL had electrophoretic mobility corresponding to 24 and 100 kDa under reducing and nonreducing conditions, respectively, suggesting that in plasma, the DlRBL is present as a physiological homotetramer. DlRBL subunit transcripts revealed an open reading frame encoding 212 amino acid residues that included two tandemly arrayed CRD and an 18-residue signal sequence at the N-terminus. The deduced size of 24.1 kDa for the mature protein was in good agreement with the subunit size of the isolated lectin. The Ca^{2+}-independent agglutinating activity of DlRBL toward rabbit erythrocytes can be inhibited in the presence of rhamnose or galactose. DlRBL agglutinated gram-positive and gram-negative bacteria and exposure of formalin-killed *Escherichia coli* to DlRBL enhanced their phagocytosis by *D. labrax* peritoneal macrophages. These results suggest that plasma DlRBL may play a role in immune recognition of microbial pathogens and facilitate their clearance by phagocytosis.[22]

s0035 RBL—MOLECULAR STRUCTURE, PHYLOGENY, AND EVOLUTION

p0080 As previously described, RBLs contain variable numbers of CRDs and, therefore, can vary significantly in length. STL1, CSL1, WCL1, and SAL have three tandemly repeated CRDs, of about 95 amino acid residues, STL2, STL3, CSL2, CSL3, and WCL3 and DlRBL contain two repeated CRDs (Table 18.1).

p0085 Comparison of the amino acid sequences among CSLs show 42–52% identity, while CSLs show 94–97% sequence identity when compared with the corresponding three RBLs from the steelhead trout eggs, STL1, STL2, and STL3. Moreover, CSL1, CSL2, and CSL3 are formed by 4, 18, and 2 subunits, respectively, interacting via noncovalent binding. The crystal structure of CSL3 revealed that it is a homodimer of two 20 kDa subunit and forms a pseudo-tetrameric structure[51]; a tetrameric conformation has also been described in *D. labrax*.[22] The detailed phylogenetic analysis of the CRDs in RBLs showed highly conserved sequences in their N-CRDs or C-CRDs indicating a probably ancient CRDs duplication. In contrast, the N and C-CRD from the echinoderm *S. purpuratus* and the urochordate *C. intestinalis* clustered together indicating a closer similarity between their C- and N-CRDs and a more recent origin of this duplication. The homology model of DlRBL based on the *O. mykiss* CSL3 RBL structure (40.31%, of identity E value 0.00e-1) showed substantial structural overlap.[22]

[AU1]

p0090 The gene organization of the fish RBL suggests that the RBL ancestral gene may have diverged and evolved by exon shuffling and gene duplication,

producing functionally diversified forms in different organisms. According to Ogawa et al.,[36] animal RBL CRDs were clustered into seven groups. The composite CRD structure of RBLs allowed the identification of 13 types of RBL genes. The seven CRDs were used to classify each channel catfish RBL constituent CRD. As shown in Table 18.1, three RBL genes containing three CRDs (CRD5-3-3, in an N–C orientation) were classified as type Ia. Two RBL genes composed of two CRDs (CRD5-3) were a new type named IIIg. The RBL containing only one CRD3 domain was termed type Va.

s0040 FUCOSE-BINDING LECTINS

p0095 FTLs constitute the most recently identified lectin family, characterized by a unique amino acid sequence motif and structural fold, and a nominal specificity for L-Fucose.[20] Unlike CTLs, Ca^{2+} is required for structural stabilization, rather than participating in direct cation–saccharide interactions.[28] FTLs (Table 18.2) have been identified in the serum from fish.[20] While the European eel (*A. anguilla*) agglutinin (AAA)[20,28] possesses a single CRD, those from the striped bass (*Morone saxatilis*), MsFBP32,[20] the sea bass (*D. labrax*), DlFBL,[23,30] and the sea bream (*Sparus aurata*), SauFBL,[31] exhibits two tandemly arrayed (N- and C-terminal) CRDs. The structure of MsFBP32 in complex with L-fucose revealed a trimeric organization with two globular opposite halves containing respectively the N-CRDs and the C-CRDs.[28] We proposed that fish F-lectins mediate immune defense responses both in the bloodstream[20,28,29] and the intestinal mucus.[20,23,30,31] They are expressed in larval and juvenile tissues and are also stored in eggs.[33]

p0100 The scarcity of bacteria possessing FTL CRDs suggests that it may have been acquired through horizontal transfer from metazoans.[20] The absence of the FTL CRD in higher vertebrates is an evolutionary enigma that coincides with land colonization after cleidoic egg appearance.[20] [AU2]

s0045 FISH FTLs: BIOCHEMICAL AND MOLECULAR FEATURES

p0105 In fish FTLs, the F-type CRD can be present either as a single CRD, or as tandemly arranged F-type CRD repeats.[20] In most teleosts, FTLs contain either duplicate or quadruplicate (steelhead trout) tandemly arrayed F-type CRDs yielding subunits of variable sizes, even within a single fish species.[20] The multiple duplicate tandem homologues present in modern teleost orders appear to be the product of independent duplications.[20]

p0110 Previously, we reported the purification of sea bass (DlFBL) and gilt head bream (SauFBL) serum fucose-binding lectins,[23,30,31] and we also demonstrated that SauFBL displays epitopes recognized by anti-DlFBL–specific antibodies. Furthermore, based on the DlFBL cDNA sequence, we showed that it is a bona fide FTL, as it shares carbohydrate specificity and biochemical properties with other well-characterized FTLs.[23] FTLs have also been described in the shark

t0015 **TABLE 18.2** Classification of FTL Family Lectins Isolated From Teleostean Fish Species

Species	Order	F-Type Lectin	F-Type CRD	Recognition Specificity	Structure	No. of CRD	References
Anguilla japonica	**Anguilliformes**	Eel fucolectin			Two disulfide-linked dimers		17
Anguilla anguilla	**Anguilliformes**	AAA	▭	L-Fucose; D-galactose; H and Le^a antigens	Homotrimer	1	23
Morone saxatalis	**Perciformes**	MsaFBP32	▬▭	Fucosilated oligosaccharides	Cylindrical trimer	2	16
D. labrax	**Perciformes**	DlFBL	▬▭	L-Fucose; galactose; melibiose; lactulose	Cylindrical trimer	2	41,20
Sparus aurata	**Perciformes**	SauFBP32	▬▭	L-Fucose; galactose; melibiose; lactulose	Cylindrical trimer	2	44
Oreochromis niloticus	**Perciformes**	TFBP	▬▭	Fucose	–	2	48
Lateolabrax japonicus	**Perciformes**	JspFL	▬▭	Fucose	–	2	92
Aristichthys nobilis	**Cypriniformes**	GANL	▬▭	Fucose	Homomultimeric	2	47
Danio rerio			▬▭			2	16

CRD, carbohydrate recognition domain.

[AU6]

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Scylorhinus canicula,[34] and other fucose-binding lectins have been identified in bighead carp (*Aristichthys nobilis*),[52] Nile tilapia (*Oreochromis niloticus* L.),[53] and the Antarctic fish (*Trematomus bernacchii* and *Dionotraco hamatus*) (unpublished data) although the lack of full sequence information in these two species has prevented their identification as members of the FTL family. [AU3]

p0115 The crystal structures of the single-CRD FTL from *A. anguilla* (AAA) and the binary CRD FTL from *M. saxatilis* (MsFBP32) have revealed that the FTL fold consists of a jellyroll β-barrel topology.[28,29] In MsFBP32, although the overall structure of the N-CRD is highly similar to that of the C-CRD, significant differences in the topology of the N- and C-CRDs were identified, particularly in those features corresponding to the extended binding site that surround the primary recognition site where L-fucose binds.[29] These differences strongly suggest that the N-CRD recognizes more complex fucosylated oligosaccharides and with a relatively higher avidity than the C-CRD.[29,16] These results have suggested that the individual CRDs of *Ms*FBP32 and other binary CRD F-lectins can bind to ligands on the microbial or host cell surface supporting the role FTLs as opsonins. As the single-CRD AAA can form dimers, it is also possible that recognition of topologically similar ligands on the microbial and host cell surfaces can also lead to opsonization.[16] Exposure of bacteria to the FTLs, DlFBL and SauFBL, enhanced phagocytosis[23,31] and supports the notion that these lectins mediate innate immune functions as opsonins by cross-linking microbial pathogens to phagocytic host cells.[16,29] Variability of critical residues in the binding pocket and surrounding loops in the multiple isoforms[28] expressed in the Japanese eel FTL[15] suggests that alternative interactions with terminal and subterminal sugars may expand the range of diverse oligosaccharides recognized by the lectin isoform repertoire.[16,28,29]

s0050 FTLs—PHYLOGENY AND EVOLUTION

p0120 Similarity searches in genomic databases revealed that the FTL sequence motif is phylogenetically broadly distributed, being present in both lophotrochozoan (ie, molluscs and planaria) and ecdysozoan protostomes, in echinoderm, in a cartilaginous vertebrate and in both early branching clades of vertebrates, lobe-finned and ray-finned fish.[20] A large number of FTLs with diverse domain topologies were identified in a variety of taxa from prokaryotes and invertebrates to amphibians, such as the *Streptococcus pneumoniae TIGR4*, the "furrowed receptor" and CG9095 of *Drosophila melanogaster*, the *Xenopus laevis* pentraxin 1 fusion protein, *Microbulbifer degradans* ZP 00065873.1, and yeast allantoicases.[20] Further, Bianchet et al.[28] described that the FTL fold is widely distributed in other proteins even with lower sequence similarities, for example, C1 and C2 repeats of blood coagulation factor V, C-terminal domain of sialidase, N-terminal domain of galactose oxidase, APC10/DOC1 ubiquitin ligase, and XRCC1. Interestingly, in modern teleosts (ie, striped bass, zebrafish,

steelhead trout, stickleback, pufferfish, and sea bass), the predominant arrangement is either duplicate or quadruplicate tandem F-type domains. Clearly, the F-type fold, favors the formation of concatenated CRD topologies in numbers that appear lineage related.[20] It is noteworthy, however, that the FTL CRD sequence motif has not yet been identified in genomes of higher vertebrates such as reptiles, birds, and mammals.[20]

s0055

GALECTIN STRUCTURE AND EVOLUTION

p0125 Galectins, the most conserved and ubiquitous lectin family detected from protists to mammals, are characterized by their specificity for β-galactosides (such as lactose and N-acetyllactosamine), a lack of Ca^{+2} requirement for ligand binding, and the presence of a conserved sequence motif in the CRD.[54] From the structural standpoint, galectins are defined by a β-sandwich structure formed by six (S) and five (F) strand sheets, with the S4–S6 strands containing the carbohydrate-binding amino acid residues.[54] The first galectin was identified and characterized from the electric organs of the electric eel *Electrophorus electricus*,[55] and since then members of the galectin family have been found in mammals, birds, amphibians, fish, nematodes, sponges, and some fungi.[1,56,57]

p0130 Based on their domain organization, mammalian galectins have been classified in three types: "proto," "chimera," and "tandem-repeat."[1] Prototype galectins contain one CRD per subunit and are usually homodimers of noncovalently linked subunits.[1] The homodimer is necessary for the binding and signaling on the cell surface.[57–59] The chimera-type galectins have a C-terminal similar to the prototype and a non-CRD N-terminal domain rich in proline and glycine. The N-terminal domain with collagen-like sequences in the presence of multivalent carbohydrate ligands could result in oligomerization.[60] Garner and Baum[61] proposed a model in which cell function may be "fine-tuned" by galectin binding. Tandem repeat galectins, in which two CRDs are joined by a linker peptide, are monomeric with a constitutive bivalency.[59]

p0135 Although the number of genes is different in various species, the galectin structures are well conserved among invertebrates and vertebrates.[57,62,63] They play important roles in morphogenesis, cell proliferation, cell death, tumor functions, and numerous pathological processes.[1,62–67]

p0140 A substantial number of galectins from the three different types have been identified and characterized in various tissues, plasma, and mucus of elasmobranch and teleost fish, which show structural and binding specificity conservation with mammalian counterparts.[17,27,68–70.]

p0145 Although initially shown to be involved in early development of vertebrates, galectins were later shown to participate in the regulation of immune homeostasis.[67] More recently, galectins have been demonstrated to participate in recognition of microbial pathogens.[14] Extracellular galectins exhibit various degrees of affinity interactions with glycans and form complexes with glycoprotein receptors[61] that can induce cellular responses such as proliferation, cell

adhesion, migration, cell motility, and apoptosis.[71,72] Intracellular galectins can participate in biological responses, including cell differentiation, inflammatory mediators as cytokines.[73]

p0150 Duplication and divergence events can explain the evolution of the various chordate galectins[56,62] while multiple lectin gene copies, allelic variation, or posttranslational modifications of the gene products expanded the molecular diversity and recognition capabilities.[57]

s0060 C-TYPE LECTINS

p0155 The CTLs are characterized by Ca^{2+} requirement, diverse carbohydrate specificity, and multiple structural domains sometimes forming chimeric structures.[1,12] CTLs have been classified in groups comprising collectins, proteoglycan core proteins, selectins, endocytic receptors, and the mannose-macrophage receptor, some of them directly or indirectly involved in immune function.[1,12] The collectins include the MBLs and conglutinin from serum and saliva, and the pulmonary surfactant, with critical roles in innate immunity against viruses and bacteria. Some NK cell and macrophage receptors and selectins are also CTL-related.[1,12] MBL consists of multimers of an identical polypeptide chain of 32 kDa and comprises four distinct regions: a cysteine-rich N-terminal region; a collagenous domain; a neck region, a short α-helical coiled-coil domain, and a CRD at C-terminal.[12]

p0160 MBL binds terminal D-mannose, L-fucose, and N-acetyl-D-glucosamine (GlcNAc), but does not interact with D-galactose and sialic acid. Selectivity concerns the presence of conserved amino acid residues within their CRDs. The hydroxyl groups are on C3 and C4 orientated in the equatorial plane of the pyranose ring.[12,74] The CRD is approximately 120 amino acids long along with a distinctive double loop stabilized by two conserved disulfide bridge conserved polar and hydrophobic interaction. There are four Ca^{2+} binding sites recognized in CRDs from various species, and the Ca^{2+} binding site is important with carbohydrate-binding activity.[1,12,74]

s0065 MANNOSE-BINDING LECTINS IN FISH

p0165 MBL is an important component of innate immunity capable of activating the lectin pathway of the complement system by co-opting MASPs, MBL-associated serine proteases. It acts as opsonin promoting phagocytosis of foreign material.[1,12,74] MBL is known also as an acute phase protein produced by hepatocytes and modulated by infection or during inflammatory response. In the presence of Ca^{2+}, MBLs initiate a broad range of biological processes such as adhesion, endocytosis, complement activation, and pathogen neutralization.[1,12,18,74]

p0170 MBLs have been reported in several fish species (reviewed in Vasta et al.[17]). The first lectin was isolated from the serum of the Atlantic salmon (*Salmo salar*) by mannose-affinity chromatography; its structure and immune functions were

also characterized.[75] It exhibited antibacterial activity and could enhance macrophage activity.[25,75]MBLs were also purified from rohu (*L. rohita*), rainbow trout (*O. mykiss*), sea lamprey (*Petromyzon marinus*), common carp (*Cyprinus carpio*), fugu (*T. rubripes*), and turbot (*Scophthalmus maximus*).[76–80] Moreover, MBL gene from channel catfish (*Ictalurus punctatus*) was upregulated following the exposure to gram-negative pathogens.[80] In the African catfish, *Clarias gariespinus*, MBL exhibited antimicrobial activity and in tilapia (*O. niloticus*), it induced cytochine production.[81]

p0175 In the Japanese flounder (*Paralichthys olivaceus*), CTLs are expressed in the liver[82] whereas in other fish species the presence of lectins in ski and gut mucus is particularly intriguing with regards to their potential biological role(s) in external defense against microbial pathogens. CTLs (MBL-like CTLs) are present in skin mucus of the Japanese eel (*Anguilla japonica*)[83] and in the conger eel (*Conger myriaster*).[84] In the latter, conCL-s is expressed in club cells of [AU4] external and internal mucosal tissues.[84]

p0180 In healthy fish, MBL is expressed almost exclusively in the liver. In *O. mykiss* and *C. carpio*, MBLs are expressed in liver and spleen.[85–88] In *T. rubripes*, the mannose-specific lectin pufflectin gene is transcribed in gills, oral cavity wall, esophagus, and skin. Only an isoform was detected in the intestine.[89]

s0070 MBL PHYLOGENY AND EVOLUTION

p0185 MBLs are highly diversified and widely distributed in animal kingdom including fish as unique structural motifs and functional domains, regardless of whether they possess the sugar-binding properties.[12] Bony fish MBL shows the same carbohydrate specificity of human MBL. A similar, MBL molecule associated with MASP-A has been identified in the lamprey, involved in C3 system activation.[90]

p0190 It is likely that the MBLs have evolved before the emergence of agnathans and they have been conserved as a recognition molecule of the lectin pathway throughout vertebrate evolution. MBL homologues should be present in species evolved from the common ancestor of both jawed and jawless fish.[91]

p0195 Moreover, the two forms of the mammalian MBL, MBL-A, and MBL-C, must have diverged after the common ancestor of tetrapods separated from bony fish. The MBL-A/MBL-C duplication was probably an independent event in the mammalian lineage.[77] The three identified and partially characterized rainbow trout MBL homologues represent structural homologues of mammalian MBLs and are expressed in various tissues including the anterior intestine, the liver, and spleen. A rainbow trout MASP-3 homologue was also expressed in partially overlapping tissues.[92]

p0200 The polymorphic nature of MBL genes in humans is well described. Similarly, recent research demonstrated that there are multiple copies of the MBL gene in zebrafish, and that the polymorphism and copy number variation of MBL can significantly affect resistance to pathogen infection.[76] Multiple

homologues of MBL also exist in rainbow trout and common carp.[73,77] In catfish, only a single copy gene in the genome is present.[94]

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BALLARIN: 18

Non-Print Items

Abstract
Lectins are sugar-binding proteins widely distributed among animals, plants and microbial taxon, involved in diverse biological processes. In both invertebrates and vertebrates, they play key roles in non-self recognition and immune responses, such as non-self recognition, inflammatory processes, and immunomodulation. In fish, many lectin families have been identified so far and their tissue-specific expression and localization of the various lectin repertoires and their ligands is consistent with their distinct biological roles in innate and adaptive immunity. Here, we discuss the involvement of F-type lectins, rhamnose-binding lectins, galectins, and C-type lectins in pathogen recognition and opsonization through the binding of endogenous and exogenous ligands, and their additional effector roles, such as complement activation and regulation of immune functions. These lectin families, identified and characterized in fish, appear to be involved in non-self recognition, inflammatory reaction, and immunomodulation processes. Function and phylogenetic history of these lectins families are also described.

Keywords: Evolution; Fish; Fucose-binding lectin; Galectin; Immunity; Lectin; Mannose-binding lectin; Rhamnose-binding lectin.